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Reinstatement of *Leuctra biellensis* Festa, 1942 (Plecoptera, Leuctridae)

Gilles Vinçon[1], Louis Boumans[2], Jean-Luc Gattolliat[3,4]

1 *55 Bd Joseph Vallier, F38100 Grenoble, France*
2 *Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, 0318 Oslo, Norway*
3 *Museum of Zoology, Palais de Rumine, 1014 Lausanne, Switzerland*
4 *Department of Ecology and Evolution, Biophore, University of Lausanne, 1015 Lausanne, Switzerland*

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Corresponding author: *Gilles Vinçon* (gvincon@gmail.com)

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Abstract

Both molecular and morphologic characters support the reinstatement of *Leuctra biellensis* Festa, 1942 as a valid species distinct from *Leuctra nigra* (Olivier, 1811). Genetic distances between *L. biellensis* and the different populations of *L. nigra* are around 9%, while intraspecific distances among *L. nigra* haploclades are less than 1%. Morphologically, the two species can be separated in male adult specimens by the shape of the two teeth on tergite VIII, by the lateral edges of tergites and by the distal expansion of the paraprocts. *Leuctra biellensis* occurs on the southern slope of the Alps in Italy and Switzerland (Ticino and Graubünden), while *L. nigra* has a wide distribution in Central and Northern Europe. As the type material of *L. biellensis* was lost, and to avoid future confusion between the two species, we designate as neotype a male imago collected at the type locality.

Introduction

The SwissBOL (Swiss Barcode of Life) is an ongoing Project, started in 2011, which aims at inventorying the genetic biodiversity of all taxa occurring in Switzerland. Against the background of global warming and other anthropogenic pressures that are threatening freshwater aquatic biodiversity, Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) are considered as particularly vulnerable groups (Tierno de Figueroa et al. 2010, Conti et al. 2014, Errochdi et al. 2014). Between 2013 and 2015, DNA barcode sequences were obtained for 90 of the 112 stonefly species reported from Switzerland (Gattolliat et al. 2016). Complementary field searches were done in 2015 and 2016 to elucidate several cases of isolated specimens on the gene tree. First results showed high intraspecific genetic distances between some populations preliminary identified as *Leuctra nigra* (Olivier, 1811). It was hypothesized that two species were involved, one in the North of the Alps and one in the South (Gattolliat et al. 2016).

Leuctra biellensis Festa, 1942 was originally described from Val Chiobbia in the Piedmont, Northern Italy. This taxon was subsequently considered as a junior synonym of *L. nigra* by Consiglio (1967). The present reinstatement of *L. biellensis* is based both on genetic evidence and on distinctive morphological characters shared by all the specimens occurring along the southern slope of the Alps. New detailed comparative descriptions are given for *L. biellensis* and *L. nigra*.

The original description of *Leuctra biellensis* was based on a single male imago collected by F. Capra. Type material was reportedly deposited at Museo Civico di Storia Naturale "Giacomo Doria", Genova, Italia

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(Festa 1942). However, we were unable to locate the type. According to the present curator of the entomological collection, it may be lost or may have never been deposited (Maria Tavano, comm. pers. 2015). In order to stabilize the nomenclatural concept of *L. biellensis* and to avoid future confusion between *L. biellensis* and *L. nigra*, we designate a neotype for *L. biellensis,* collected as close as possible to the type locality (article 75.3, ICZN 1999). The neotype and some of the topotypes were deposited at the Museo Civico di Storia Naturale "Giacomo Doria", Genova, Italy.

Material and methods

Molecular study: DNA was extracted from specimens stored in the collection of the Museum of Zoology in Lausanne, using a non-destructive method allowing *a posteriori* morphological identification. 658 bp of the mitochondrial protein-coding gene cytochrome c oxydase subunit I (CO1) was amplified using the primers LCO1490 and HCO2198 (for details see Gattolliat et al. 2016).

Additional sequences were downloaded from the Barcode of Life Data System (BOLD) database, notably four sequences from Bavaria, projects Barcoding Fauna Bavarica (Hendrich et al. 2010) and Germany Malaise Trap 05 [GMGRC] (Geiger et al. 2016), two Belgian and four Norwegian sequences from the project 'Norwegian Barcoding of Life [NorBOL] – Freshwater Insects' (Boumans and Brittain 2012), and one more Swiss sequence from the project 'West Palaearctic Plecoptera [WPPLE]'. All specimens are listed in Table 1. Sequences of *Leuctra hippopus* Kempny, 1899 and *Leuctra pseudorosinae* Aubert, 1954 were added to the data matrix as outgroups, as these have the most similar mitochondrial haplotypes according to Gattolliat et al. (2016).

The final data matrix included 25 COI sequences of 587–658 bp (no gaps or missing data). Analyses were conducted in MEGA7 (Kumar et al. 2016). We used uncorrected p distances to calculate genetic distances between haplotypes, and within and between major haploclades (Srivathsan and Meier 2012).

Tree topology was reconstructed using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches (bootstrap, 1000 replicates).

Morphology: Ethanol-preserved specimens of *L. nigra* and potential *L. biellensis* from the collections of the Museum of Zoology in Lausanne (MZL), Delmastro (Del), Murányi (Mur), Ravizza (Rav) and Vinçon (Vin) were examined. Since 2016, all of Ravizza's collection is housed in the MZL. Neotype, as well as five male and five female imagoes are deposited at the Museo Civico di Storia Naturale "Giacomo Doria", Genova, Italy. The full list of examined specimens is given in the results section.

Results

Molecular study

The mitochondrial phylogeny clearly recovers *L. nigra* and *L. biellensis* as distinct monophyletic clades (bootstrap of 100%), with intraspecific distances below 1% (Table 2). Distances between haplotypes of *L. biellensis* and haplotypes of *L. nigra* range from 8.8 to 9.1%, while distances between these species and *L. pseudorosinae* and *L. hippopus*, are between 13 and 15% (Table 2). Distances between populations within *L. nigra* are always under 1% even in the case of geographically distant sites such as Finnmark in Norway and Bavaria in Germany (Table 2).

Morphological re-examination and redescription

***Leuctra nigra* (Olivier, 1811)**

Figs 1, 2a–g

Nemoura nigra Olivier 1811: 186
Leuctra acuminata Bengtsson, 1933 (syn. fide Brinck 1949).
Leuctra nigra Oliv., Mosely 1932: 27, figs 39–40, pl. V/26; Kühtreiber 1934: 76, fig. 55; Despax 1951: 140, fig. 61c-d; Illies 1955: 68, fig. 61; Aubert 1959: 65, figs 159–160; Mendl 1968: 70; Kis 1974: 87, 88, figs 37a–e; Ravizza and Vinçon 1998: 303, fig. 6a–f; Teslenko and Zhiltzova 2009, 223, figs 1235–1239; Lubini et al. 2012: 180, fig. 433, 196, fig. 477.
Locus typicus: France, Versailles.

Material examined. FRANCE: BRITANY: Finistère: Rosnoen, Toulencoat, 22.03.2001, 6♂ (Manach leg). **VOSGES MASSIF: Vosges:** SW Colmar, > Wasserbourg, Krebsbach, Fecht trib., Ill trib., 700 m, 17.05.1995, 10♂, 13♀; Ballon d'Alsace, Saut-de-la-Truite, 23.08.1996, 1♂, 8♀ (Vin). **MASSIF-CENTRAL: Saône-et-Loire:** Morvan Massif, Haut-Folon, 800 m, 14.07.1994, 5♂, 7♀ (Vin); **Puy-de-Dôme**: Forez Mountains, > Vertolaye, > Chansert Pass, Jasserie de la Croix Fossat, 1500 m, 17.07.1994, 3♂, 7♀; St Pierre-la-Bourlhonne, cross road to the Chansert Pass, Dore trib., 17.07.1994, 1♀; W. Clermont-Ferrand, between Pontaumur and La-Forêt, b., 21.04.2009, 1♂, 5♀ (Vin). **Loire:** Mont Pilat, Graix, 900 m, 17.07.1994, 1♀; Mont Pilat, Crêt de la Perdrix, Gier b., 1400 m, 17.07.1997, 3♀ (Vin). **Ardèche**: Mont Gerbier-de-Jonc, Loire spring, 16.06.2001, 2♀; Lachamp-Rafael, Bois de Cuze, peat bog, 1350 m, 15.06.2001, 4♂, 6♀ (Vin). **Cantal**: Plomb-du-Cantal Massif, > Super Lioran, 1300 m, 30.07.2004, 3♀ (Vin). **Tarn**: NW slope of Pic de Nord, Arnette trib., Thoré trib., 850 m, 1.05.1991, 4♂, 2♀ (Vin). **Aude**: > Citou, spring, trib. of Argent-double, 900 m, 1.07.1989, 1♀, 1.05.1991, 20♂, 3♀ (Vin). **JURA MASSIF: Ain:** La Vattay Ski Station, Faucille Pass, 1300 m, 30.05.1991, 1♂, 27.06.1995, 1♀, 13.07.1996, 1♀ (Vin); Valserine, Combe de Mijoux, Le Planet, 1200 m, 19.07.1991, 3♀; Valserine, Joux Verte, 19.07.1991, 2♀ (MZL); **Doubs**: Jougne, Swiss Border, Les Tavins, La Tavine, Orbe trib., 900 m, 28.06.1995, 1♀ (Vin). **ALPS: Haute-Savoie:** Montet Pass, > Vallorcine, Eau-Noire trib., 1450 m, 21.06.1991, 2♂, 15♀ (Vin); **Savoie**: Allevard, Val Pelouse, 1400 m, 7.06.1987, 10♂, 1♀; Saisie

Table 1. Specimens used for the phylogenetic analysis of the mitochondrial gene CO1.

Species	Sample ID	Collectors	Date	Country	Province	Exact Site	Lat	Lon	Elev	Accession number
Leuctra biellensis	LN_TI_15	P. Baumann	5/30/2007	Switzerland	Graubun-den	Cavagliasch, Cavaglia	46°21'45"	10°02'43"	1690	GBIFCH00238522
Leuctra biellensis	LN_TI_16	P. Baumann	5/30/2007	Switzerland	Graubun-den	Cavagliasch, Cavaglia	46°21'45"	10°02'43"	1690	GBIFCH00238523
Leuctra biellensis	LN_TI_17	H. Vicentini	6/2/2006	Switzerland	Ticino	Quelle Vignino, Montagnola	45°58'06"	8°55'03"	460	GBIFCH00238524
Leuctra biellensis	LN_TI_18	H. Vicentini	6/2/2007	Switzerland	Ticino	Quelle Vignino, Montagnola	45°58'06"	8°55'03"	460	GBIFCH00238525
Leuctra biellensis	LN_TI_19	H. Vicentini	6/19/2010	Switzerland	Ticino	Quelle Pian Sagno Acquacalda	46°32'04"	8°50'41"	1665	GBIFCH00238526
Leuctra biellensis	EPT-344	R. Tester Ryf	6/1/2009	Switzerland	Ticino	Ticono, Mte. Ceneri	46°08'11"	8°54'04"	680	GBIFCH00277633
Leuctra hippopus	EPT-321	S. Knispel	4/7/2010	Switzerland	Fribourg	Ruisseau, Villargiroud	46°41'22"	6°59'39"	795	GBIFCH00280313
Leuctra hippopus	EPT-322	P. Stucki	4/16/2010	Switzerland	Vaud	Veyron, La Chaux	46°37'07"	6°28'33"	540	GBIFCH00280288
Leuctra hippopus	EPT-323	V. Lubini	4/1/2009	Switzerland	Zürich	Wehrenbach, Zürich	47°21'10	8°35'02"	500	GBIFCH00280295
Leuctra nigra	NHMO-EPT_89	B. Koese & L. Boumans	3/18/2010	Belgium	Limburg	Rekem, Ziepbeek	50°55'28"	5°39'15"	50 ca	KY250738
Leuctra nigra	NHMO-EPT_80	B. Koese & L. Boumans	3/18/2010	Belgium	Limburg	Rekem, Ziepbeek	50°55'28"	5°39'15"	50 ca	KY250735
Leuctra nigra	NHMO-EPT_55	L. Boumans	5/12/2010	Norway	Hedmark	Galten, bekk til Galthaen	61°53'54	11°46'27"	655	KY250740
Leuctra nigra	NHMO-EPT_1553	T. Ekrem	7/20/2010	Norway	Finnmark	Sør-Varanger, Sameti, Sameti-johka	69°24'04"	29°43'09"	45	KY250739
Leuctra nigra	NHMO-EPT_982	L. Boumans, T. Ekrem	7/28/2010	Norway	Finnmark	Lebesby, Kunes, Austerelva	70°20'37"	26°31'09"	10	KY250741
Leuctra nigra	M200612	G. Vincon	6/20/2010	Switzerland	Jura	Jura Massif, Tramelan, Gruere lake tributaries	47°14'17"	7°03'01"	1000 ca	KY250737
Leuctra nigra	NHMO-EPT_462	L. Boumans	5/26/2010	Norway	Oppland	O Heimdalsvatn, stream to outflow	61°53'54"	8°53'51"	1095	KY250736
Leuctra nigra	EPT-342	S. Knispel	5/18/2010	Switzerland	Vaud	Affl. Bressonne, Les Censières	46°34'24"	6°42'50"	865	GBIFCH00277517
Leuctra nigra	EPT-343	V. Lubini	5/21/2009	Switzerland	St-Gallen	Tüfenbach, Tüfi	47°19'21"	9°12'44"	800	GBIFCH00277566
Leuctra nigra	BC_ZSM_ AQU_00196	M. Hess, U. Heckes & M. Franzen	7/3/2009	Germany	Bavaria	Vorderer Bayerischer Wald	48.909	13.011	900	HM421988
Leuctra nigra	BC_ZSM_ AQU_00747	M. Hess & U. Heckes	7/16/2009	Germany	Bavaria	Mangfallgebirge	47.687	12.056	990	HQ563171
Leuctra nigra	BC_ZSM_ AQU_001124	M. Hess & U. Heckes	4/10/2011	Germany	Bavaria	Hinterer Bayerischer Wald	48.94	13.41	770	KY261279
Leuctra nigra	BIOUG03760-E10	G. Sellmayer	6/8/2012	Germany	Bavaria	Bayerischer National Park	48.9509	13.422	840	KY261521
Leuctra pseu-dorosinae	EPT-532	J.-P. Reding	5/26/2014	Switzerland	Vaud	Ste Croix, Affluent Dénériaz, la Merla	46°50'55"	6°31'33"	1200	GBIFCH00280264
Leuctra pseu-dorosinae	EPT-533	J.-P. Reding	5/26/2014	Switzerland	Vaud	Ste Croix, Affluent Dénériaz, la Merla	46°50'55"	6°31'33"	1200	GBIFCH00280174
Leuctra pseu-dorosinae	EPT-534	J.-P. Reding	5/26/2014	Switzerland	Vaud	Ste Croix, Affluent Dénériaz, la Merla	46°50'55"	6°31'33"	1200	GBIFCH00279996

Table 2. Estimates of evolutionary divergence between geographical clusters of *Leuctra nigra* and *Leuctra biellensis* using uncorrected p distances. Minimum and maximum distances are indicated in brackets.

	***L. nigra* Germany & CH**	***L. nigra* Norway**	***L. nigra* Belgium**	***L. biellensis***	***L. hippopus***	***L. pseudorosinae***
***L. nigra* Germany & CH**	0.000					
	(0.000–0.001)					
***L. nigra* Norway**	0.007	0.001				
	(0.002–0.010)	(0.000–0.009)				
***L. nigra* Belgium**	0.004	0.004	0.000			
	(0.000–0.007)	(0.002–0.009)	(0.000)			
L. biellensis	0.091	0.088	0.091	0.001		
	(0.090–0.094)	(0.085–0.090)	(0.090–0.092)	(0.000–0.002)		
L. hippopus	0.141	0.139	0.141	0.135	0.000	
	(0.140–0.143)	(0.138–0.140)	(0.141)	(0.135)	(0.000)	
L. pseudorosinae	0.154	0.152	0.155	0.128	0.133	0.001
	(0.153–0.157)	(0.152–0.153)	(0.155)	(0.124–0.131)	(0.131–0.136)	(0.000–0.007)

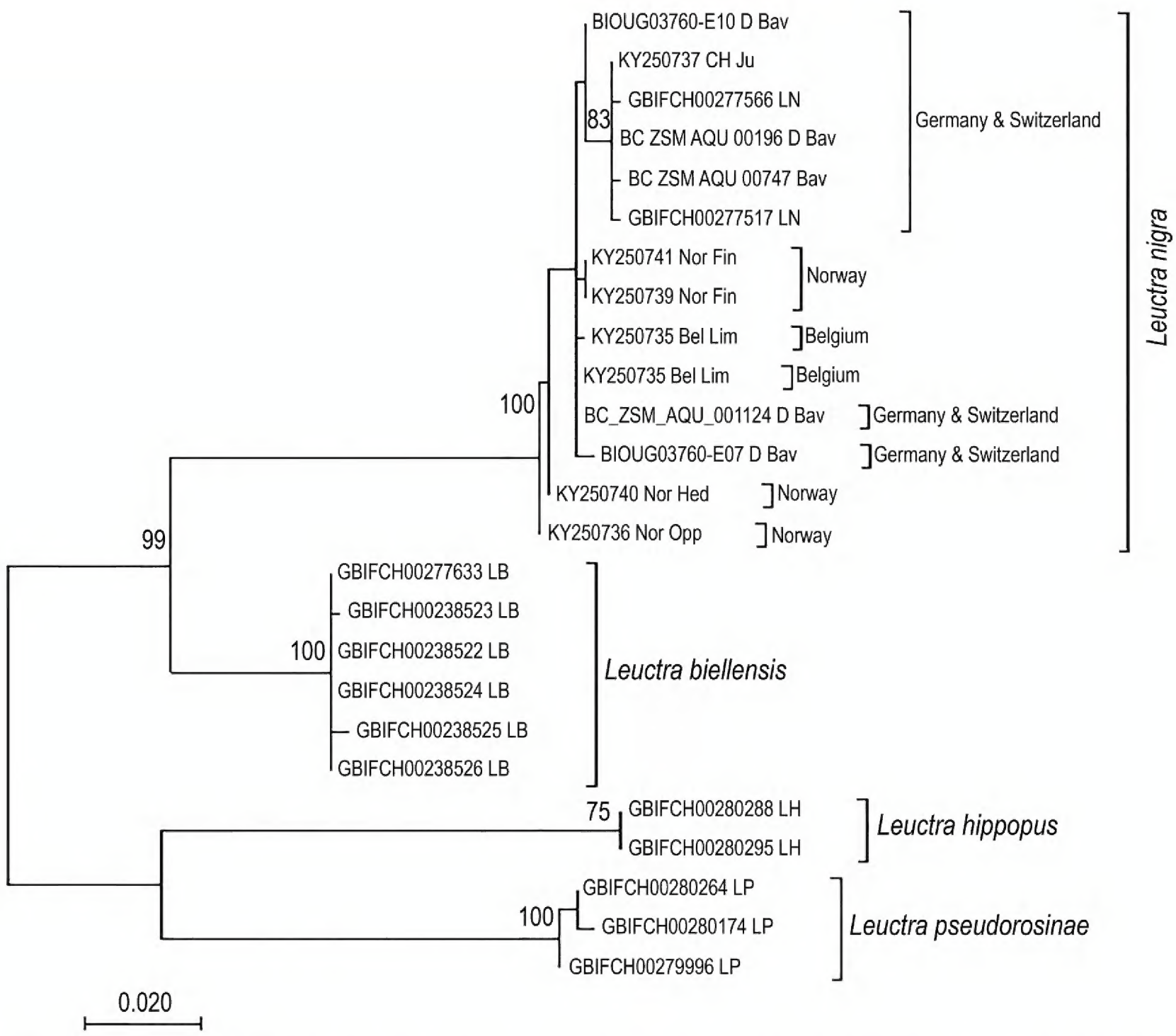

Figure 1. Maximum Likelihood (ML) consensus tree reconstructed for 25 specimens of *Leuctra* spp. Tree drawn to scale, branch lengths measured in number of substitutions per site, deeper nodes labelled above branches with Maximum Likelihood bootstrap support.

Pass, Nant Rouge trib., 1600 m, 22.05.1991, 1♂, 1♀ (Vin). **Isère**: Chartreuse Massif, < Charmette Pass, Tenaison b., Vence trib., 1200 m, 28.05.1988, 1♂ (Vin); Belledonne Massif, below Oursière Cascade, Doménon trib., brook, 1500 m, 4.06.1995, 1♂, 1♀; < Chamrousse, Fénérieux b., Prémol trib, between Prémol Forest House and Luitel Lake, 1200 m, 10.06.1985, 1♂, 3♀; < Chamrousse, Premol Forest House, Sonnant trib., 1100 m, 10.06.1985, 1♀,

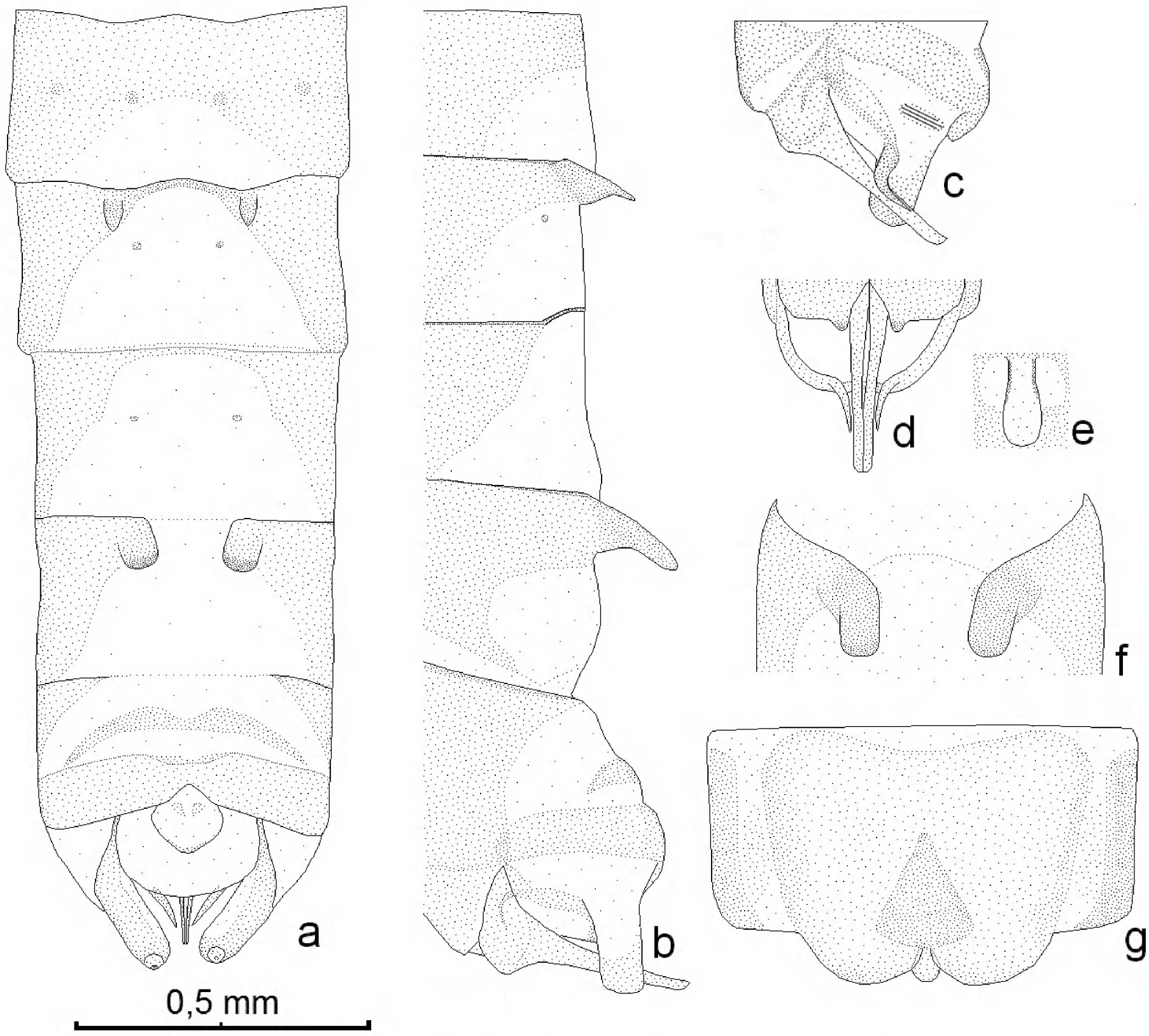

Figure 2. *Leuctra nigra*, from France, Allevard, Val Pelouse. Male: **a** = abdomen tip, dorsal view, **b** = lateral view; **c** = paraprocts lateral view, **d** = ventral view; **e** = ventral vesicle; **f** = tergite VIII top view. Female: **g** = subgenital plate, ventral view.

24.05.1998, 1♀, 19.05.2013, 7♂, 5♀, 2.06.2013, 2♂, 1♀ (Vin). **SWITZERLAND, JURA MASSIF**: NW Tramelan, Gruère Lake, b. right side of the lake, 20.06.2012, 5♂, 11♀ (Vin). **GERMANY**: Fulda, 1981, 2♂, 1♀ (Zwick leg), **SLOVAKIA**: Vysoké Mountains, Tatry, Podspady, Javorinka r., 11.07.1967, 5♂, 5♀ (Steinmann & Mur).

Complementary description. Based on French specimens: Savoie, Allevard, Val Pelouse, 1400 m, 7.06.1987. **Male** (Fig. 2a–f): Tergite VI with two small triangular appendices pointing upwards and backwards in side view (Fig. 2b). Tergite VII with non-interrupted anterior margin and wide median bell-shaped membranous area. Tergite VIII with two strong teeth pointing upwards and backwards in side view (Fig. 2a, b, f); teeth sub-rectangular in dorsal view (Fig. 2f). Tergite IX with anterior margin interrupted on nearly half segment width, lateral edges triangular ending in sharp angles near anterior margin. Lateral lobes of paraprocts with a sclerotized hook-shaped expansion turning backwards and extending along distal part of specilla (Fig. 2c–d). Specilla straight in ventral and side views (Fig. 2c–d), slightly curved at their tip (Fig. 2c). Sternite IX: ventral vesicle racket-shaped (Fig. 2e). **Female** (Fig. 2g): subgenital plate with two rounded lobes separated by a small rounded lamella; a triangular sclerite placed between the 2 lobes is visible beneath transparent cuticle.

Leuctra biellensis Festa, 1942

Figs 1, 3a–g, 4

Leuctra biellensis, Consiglio 1962

Leuctra nigra Oliv., Fochetti and Tierno de Figueroa 2008: 259, fig. 160a–f.

Locus typicus: Alpe Finestre, Val Chiobbia, Piemonte (altitude 1700 m).

Material examined. Neotype (GBIFCH00235761): Italy, Pennine Alps : Biellese mountains, Oropa, torrents and brooks, 1200–1900 m, 06.1978–07.1978, Coll. C.

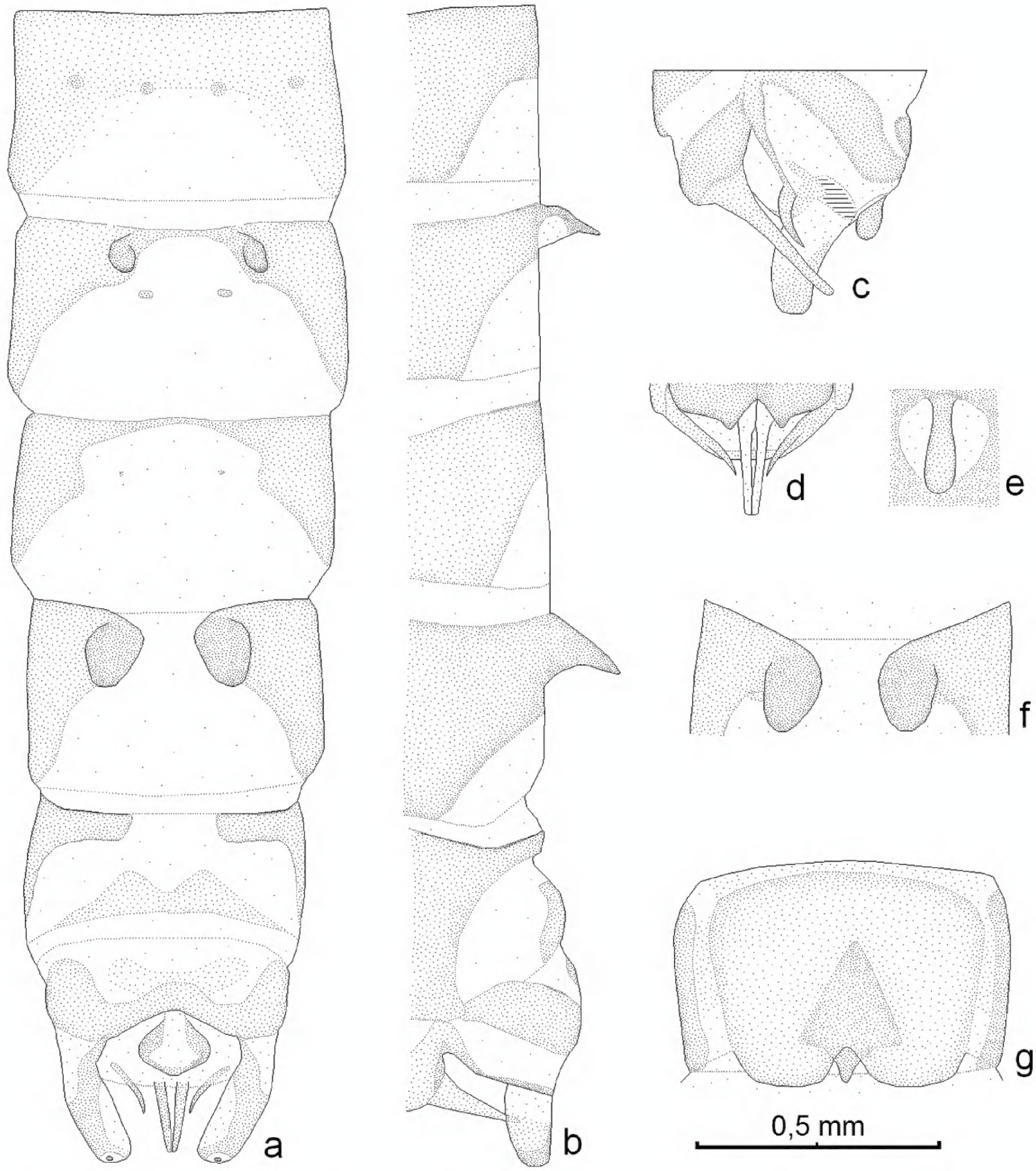

Figure 3. *Leuctra biellensis* from Swiss, Ceneri Mount, Isone. Male: a = abdomen tip, dorsal view, b = lateral view; c = paraprocts lateral view, **d** = ventral view; **e** = ventral vesicle; **f** = tergite VIII top view. Female: **g** = subgenital plate, ventral view.

Ravizza, 1♂ (Museo Civico di Storia Naturale "Giacomo Doria", Genova, Italy).

Topotypes (GBIFCH00235762): same data as neotype: 175♂, 153♀.

ITALY: Pennine Alps: Civiasco, (NO), 700 m, 25.04.1992, 16♂, 2♀; Valsesia, Morce brook, 500 m, 25.04.1975, 5♂, 1♀ (Rav); Biellese mountains, brooks, Elvo trib., 1100 m, 18.05.1981, 7♂, 1♀(Rav); Biellese mountains, Donato, Vione tor., 950 m, 21.05.1979, 16 larves (1 male nymph) (Rav); Andrate, Graglia, slow brook, Mombarone mount, 5.06.1978, ♂, ♀ (Rav). Aosta Valley, Dora Baltea trib., > Trovinasse, Colla della Lace, 1900 m, 11.07.2003, 1♂; > Trovinasse, Colla della Lace, 1700 m, 24.05.2003, 4♂, 6♀; Dora Baltea trib., Pont-St-Martin, > Carema, 500 m, 1.06.1991, 2♂, 4♀; Dora Baltea trib., > Nomaglio, road from Andrate to Biella, 600 m, 1.06.1991, 13♂, 9♀; 31.03.2000, 1♂, 1♀; E. Varallo, Civiasco, Sesia trib., b., 850 m, 2.06.1991, 2♂, 1♀; N. Varallo, > Cervarolo, 1500 m, 2.06.1991, 4♂, 1♀; < Cervarolo, > Piane del Alpe, b., 1200 m, 2.06.1991, 9♂, 6♀; W. Grevellona, Val Strona, > Campello Monti, 1500 m, 13.08.2005, 1♀; Val Strona, > Massiola, < Rosarolo, b., 750 m, 26.04.1999, 12♂, 3♀; Val Strona, > Omegna, b., 500 m, 24.05.2003, 2♂, 1♀; SW. Domodossola, Valle Anzasca, Macugnaga, Staffa, b., 1300 m, 2.06.1991, 16♂, 16♀; Valle Anzasca, Macugnaga, Prequartera, 800 m, 1.06.1991, 3♂, 3♀ (Vin). **Cottian Alps:** Ceres, Almesio, 750 m, 11.06.2001, 4♂, 3♀; Lemie, Val d'Ovarda, A. Milone, 1650 m, 20.06.2001, 1♂, 4♀; Locana,

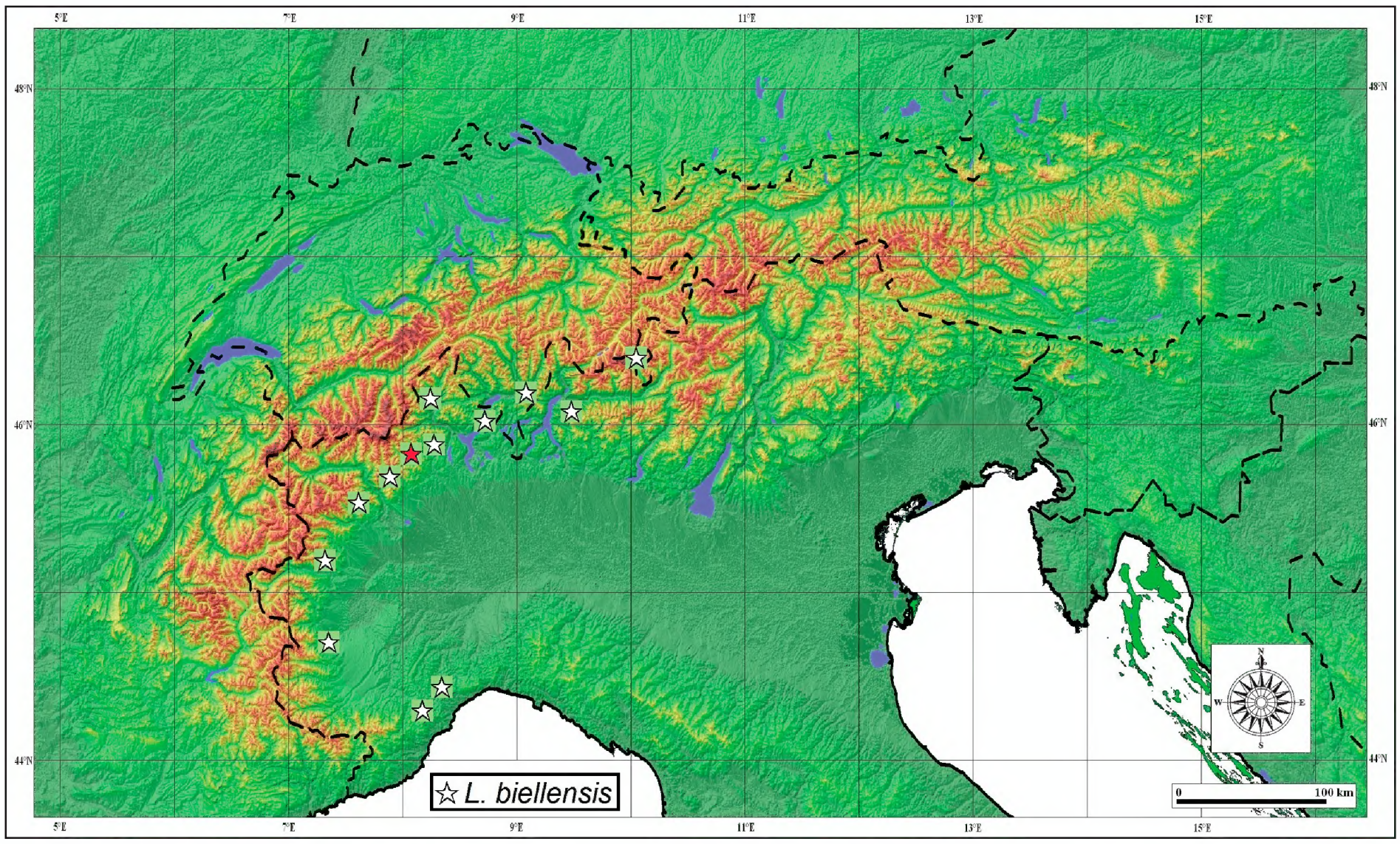

Figure 4. Distribution area of *Leuctra biellensis*. Red star = Type locality. White star = other localities with *L. biellensis*.

val Piantonetto, S. Giacomo, 1130 m, 26.06.2001, 1♀; Villafranca Piemonte, TO, Cappella della Missione, 260 m, 24.03.2002, 2♂; (Del); **Graian Alps**: Frassinato brook, Soana trib., 1000 m, 18.06.1992, 1♀ (Rav); Val Soana, 700 - 850 m, 11♂, 6♀ (Rav); Lis Pass, > Girardi, Stura di Lanza trib., b., 1100 m, 7.05.2000, 5♂, 2♀; 27.08.2005, 2♀; Val di Viu, Stura di Lanza trib., > Viu, Piazette, b., 1200 m, 1.04.2000, 3♂; Val di Viu, Stura di Lanza trib., < Viu, brook, 600 m, 22.04.2008, 3♂, 6♀; NE. Lanzo Torinese, > Corio, Pian d'Audi, Rio Malone trib., 865 m, 23.10.2000, 1♀; Aosta Valley, > Quincinetto, > Scalaro, 1500 m, 1.05.2009, 1♀; > Quincinetto, Rio della Folla, 1250 m, 11.07.2012, 1♀; > Quincinetto, road to Lecchia sup. and road to Scalaro (iron bridge), 1100 m, 1.05.2009, 1♂; Aosta Valley, > Champorcher, Valle della Legne, Ayasse trib., 1200 m, 6.05.2000, 1♂ (Vin). **Rhaetian Alps**: Po Valley, Rocchellaif, Po, 550 m, 1.05.1982, 4♂, 1♀ (Rav). **Liguria**: Calizzano brook, 900 m, 28.04.1976, 3♂ (Rav); (SV) Calizzano, Melogno Pass, Frassino trib. 800-1000 m, 27♂, 4♀ (Rav); Montenotte, Erro spring, 700-950 m, 4.05.1974, 48♂, 40♀ + 65♂, 58♀ (Rav); Mont San Giorgio, brook, 750 m, 16.04.1974, 10♂, 3♀ (Rav). **Lombardia**, Casargo, (CO), Varonne tor., 2.06.1973, 21♂, 16♀, Bucuarelli leg (Rav); PreAlps, San Michele (VA), Olocrene spring, 800 m, 8.07.1986, 2♂, 3♀(Rav).

SWITZERLAND: Ticino, Lepontine Alps: Ceneri Mount, > Isone, before the military base, 1.04.2015, 10♂, 5♀ (Vin); Valle Lucoresagguo, Piace Seguo, 1650 m, 23.05.1988, 28♂, 16♀ (Rav). **Rhaetian Alps: Graubünden,** Cavagliasch, Cavaglia, 1690 m, 30.05.2007, 2♂ (P. Baumann).

Complementary description. Male (Fig. 3a–f): Tergite VI with two small triangular appendices pointing upwards and backwards in side view (Fig. 3b). Tergite VII with non-interrupted anterior margin and wide median bell-shaped membranous area. Tergite VIII with two strong teeth pointing upwards and backwards (Fig. 3a, b) and nearly triangular in dorsal view (Fig. 3f). Tergite IX with anterior margin interrupted on nearly one third of segment width, lateral edges sub-triangular forming a wide strip along anterior margin (Fig. 3a). Lateral lobes of paraprocts with a sclerotized sickle-shaped expansion slightly bent towards distal part of specilla (Fig. 3c–d). Specilla straight in ventral and side views (Fig. 3c–d). Sternite IX: ventral vesicle racket-shaped (Fig. 3e). **Female** (Fig. 3g): subgenital plate with two rounded lobes separated by a small triangular lamella; a triangular sclerite placed between the 2 lobes is visible by transparency under the cuticle.

Ecological preferences and distribution area. *Leuctra biellensis* is a crenophilic species occurring in springs and brooklets at various altitudes (260–1900 m). The flight period is mainly in spring and early summer (III–VII) but few adults also occur in autumn (VIII–X). Its distribution area widely covers the western part of the Italian Alps from the Rhaetian Alps to the Maritime Alps and also extends in Liguria (Fig. 4). Its occurrence in Switzerland is restricted to the southern slope of the Alps.

Discussion

Aubert (1954) was the first who challenged the validity of *Leuctra biellensis*, suggesting that it could be a junior synonym of *Leuctra nigra*, but without formally establishing

the synonymy. In the same way, Illies (1966) also considered the species as doubtful but did not change its status. Consiglio (1962, 1967) likewise adopted an ambivalent attitude. He first considered *L. biellensis* to be a valid species (Consiglio 1962), then changed his mind and established the synonymy with *L. nigra* (Consiglio 1967, p. 18). This synonymy was then confirmed by Zwick (1973). However, neither Aubert nor Consiglio adduced the required morphological details that would have justified this synonymy.

Male and female imagos of these two species can be morphologically distinguished by the following characters. In the male, the two teeth of tergite VIII are sub-triangular in dorsal view in *L. biellensis* (Fig. 3f), instead of sub-rectangular in *L. nigra* (Fig. 2f); the lateral edges of tergite IX form a wide sclerotized strip on nearly half part of the anterior margin in *L. biellensis* (Fig. 3a), while they end in acute angles in *L. nigra* (Fig. 2a); the distal expansions of the paraprocts are sickle-shaped and not lying along the specilla in *L. biellensis* (Fig. 3d), while they are hook-shaped with their tip lying along the specilla in *L. nigra* (Fig. 2d). In the female, the lamella between the two lobes of the subgenital plate is triangular in *L. biellensis* (Fig. 3g) and rounded in *L. nigra* (Fig. 2g).

Leuctra biellensis is an alpine micro-endemic species only occurring in the western and internal part of the Alps like other cryptic species of Plecoptera (Ravizza and Vinçon 1998) or Trichoptera (Graf et al. 2015). The species is strongly crenophilic, with long flight period (III–X). Conversely, *L. nigra* is a central north-European species, mainly occurring in lentic biotopes and with shorter flight period (V–VIII) (Ravizza and Vinçon 1998). Both species were never collected together in the same locations and therefore *L. biellensis* can be considered as a sister species of *L. nigra*, inhabiting the internal slope of the Alps where it was probably isolated from *L. nigra*. The same applies to many other alpine species such as *L. muranyii* Vinçon and Graf, 2011 and *L. juliettae* Vinçon and Graf, 2011, two sister species of *L. braueri* Kempnyi, 1898, isolated in a restricted part of the eastern Alps (Vinçon and Graf 2011). Molecular tools associated with morphological characters are very promising to separate species in groups with recent diversification (Vitecek et al. 2017).

We found around 9% of distance between COI haplotypes of *L. nigra* and *L. biellensis*. While a 3.5% COI sequence distance has been proposed in the DNA barcoding literature as a likely maximal value for intraspecific divergence (Hebert et al. 2003; Zhou et al. 2010), higher intraspecific K2P and p values are not uncommon (e.g., Meier et al. 2006), and have also been reported for Plecoptera (Mynott et al. 2011; Boumans and Baumann 2012; Gill et al. 2015). Nonetheless, 9% distance is an unlikely intraspecific value. We are aware that species cannot be identified or described based on mitochondrial sequences alone. A major reason for this is that strongly divergent haploclades in some populations may result from hybridisation and subsequent mitochondrial introgression (Boumans and Tierno de Figueroa 2016). For this reason, we emphasise that the mitochondrial and morphological data converge to identify *L. biellensis* as a valid species distinct from *L. nigra*. Since our molecular study also contains Scandinavian samples, our results confirm the presence of *L. nigra* in this area and tend to confirm the synonymy made by Brinck (1949) of *Leuctra acuminata* Bengtsson, 1933 with *L. nigra,* especially as *L. acuminata* was established for specimens from Sweden originally identified as *L. nigra*.

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